Lipids of the Mutant Bronze Mosquito, Aedes aegypti (L.) [1]

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Abstract

There were no differences in neutral lipid or phospholipid content and composition between mutant bronze and wild-type mosquitoes, *Aedes aegypti* (L.). However, fatty acid composition within some of the lipid fractions was found to vary. The bronze mosquitoes contained more saturated and less unsaturated fatty acids than wild-type in free fatty acid, mono- and diglyceride, phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine fractions. In the sterol ester fraction the wild-type contained more C-18:1 (oleic) than C-18:2 (linoleic), while the reverse was true for bronze.

The importance of fatty acids as components of cuticular lipids, as necessary constituents for egg viability, and as integral parts of phospholipid molecules is discussed in reference to the sterility of the mutant bronze. It is hypothesized that alterations in fatty acid composition could be at least partially responsible for the failure of the bronze eggs to hatch.

Introduction

Females of the mutant bronze of the Yellow Fever Mosquito, *Aedes aegypti* (L.), are sterile because their eggs fail to hatch (Bhalla and Craig, 1967). The females mate normally and fertilization occurs but embryonic development proceeds for only about six hours after fertilization. Bhalla and Craig suggest that the absence of melanin formation and normal tanning of the egg shell might be responsible for the failure to complete embryonic development. However, when egg shells of the mutant bronze were tanned artificially with benzoquinone so they could not be differentiated from wild-type eggs, only a slight enhancement in embryonic development occurred and the eggs still failed to hatch.

A recent paper by Jackson *et al.* (1968) points out that unsaturated fatty acids may play a vital role in insect metabolism and that a rather exact fatty acid composition may be necessary for egg viability. With this in mind we have completed a study of the lipids of mutant bronze and wild-type *Aedes aegypti* in an attempt to detect alterations in lipid metabolism which might account for the failure of the eggs to complete embryonic development.

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Materials and Methods

The colonies of the two strains of *Aedes aegypti*, wild-type and bronze, were maintained using standardized rearing methods (Bhalla, 1966; Bhalla and Craig, 1967). The larval food consisted of Gaines dog food and yeast. Pupae were sexed and placed in screened cups for emergence. One to two-day-old unfed adults were used for lipid analysis.

Total lipids were extracted with chloroform-methanol, 2:1 (v/v) and purified by the method of Folch *et al.* (1957). The total lipid extract was placed on a silicic acid column and neutral lipids were eluted with chloroform, while the retained phospholipids were eluted with methanol-chloroform, 9:1 (v/v). Neutral lipids were fractionated by thin layer chromatography on silica gel plates (Merck) using a solvent system of petroleum ether-ethyl ether-glacial acetic acid, 85:15:1 (v/v/v) (Kinsella, 1966). The fractions were identified by co-chromatography with known lipids (Applied Science Labs.) and by gas-liquid chromatography. The area of silica gel containing each fraction was scraped from the plate and the lipids were eluted with chloroform-methanol, 2:1 (v/v), dried, and weighed. A portion of each fraction was quantitated by the sulfuric acid colorimetric method of Marsh and Weinstein (1966) as described previously (Yurkiewicz and Whelchel, 1969). Sterols were also quantitated by the method of Sperry and Webb (1950). No significant differences were found in the data from the two methods of sterol quantitation.

Phospholipids were fractionated on glass fiber-silica gel sheets (Gelman Instrument Co.) as reported earlier (Yurkiewicz, 1967) and an aliquot was quantitated by direct phosphorus analysis (Chen *et al.*, 1953). Identification was by co-chromatography with known phospholipids (Applied Science Labs.) and by hydrolysis of the individual fractions followed by paper chromatographic examination of the hydrolytic products (Dawson, 1960).

The lipid fractions were saponified in 10 per cent ethanolic KOH for 20 min. at 55° C. After acidification with 50 per cent HCl, the free fatty acids were removed with hexane. Fatty acid methyl esters were prepared using the boron trifluoride method of Metcalfe and Schmitz (1961) and were analyzed by gas-liquid chromatography (Yurkiewicz, 1969). Standard chromatographic techniques, including the use of diethylene glycol succinate columns and hydrogen flame detectors, were employed.

The mean values from two to three determinations on single pooled mosquito samples are presented in the tables. In those cases where standard deviation is included four determinations were made.

TABLE 1. Neutral lipid content and composition of mutant bronze and wild-type *Aedes aegypti*

Insect	Total Neutral Lipid (mg/g live wt)	Per Cent Composition				
		Tri-glyceride	Mono- + Diglyceride	Free Fatty Acid	Sterol	Sterol Ester + Hydro-carbon
Wild-type ♂	34.5 ± 3.3[1]	76.2	10.9	3.3	3.1	6.5
Bronze ♂	31.2 ± 2.7	73.7	13.4	2.8	2.9	7.2
Wild-type ♀	37.8 ± 4.7	81.4	8.2	2.5	2.3	5.6
Bronze ♀	36.1 ± 3.9	78.9	9.7	2.2	2.4	6.8

[1] Mean ± Standard Deviation.

RESULTS AND DISCUSSION

The neutral lipid content and composition of wild-type and bronze mosquitoes are shown in Table 1. No differences in neutral lipids are obvious between the two strains of mosquitoes, but females of both strains appear to contain slightly more lipid than males. Triglyceride is the major lipid fraction and accounts for over 70 per cent of the neutral lipids in all insects. This is in agreement with Van Handel and Lum (1961), who found mainly triglycerides and traces of sterols, free fatty acids, and hydrocarbons in *Aedes sollicitans*.

Phospholipid content and composition are shown in Table 2. The total phospholipid content is similar to earlier reports on *Aedes aegypti* (Khan and Brown, 1966; Fast and Brown, 1961); but the per cent composition differs because the total phospholipid sample was fractionated into

TABLE 2. Phospholipid content and composition of mutant bronze and wild-type *Aedes aegypti*

Insect	Total Phospholipid (mg/g live wt)	Per Cent Composition (lipid phosphorus)				
		Lysophos-phatidyl Choline + Sphingolipid	Phosphatidyl Choline	Phosphatidyl Serine + Phosphatidyl Inositol + Lysopho-phatidyl Ethanolamine	Phosphatidyl Ethanolamine	Cardiolipin
Wild-type ♂	6.9 ± 1.2[1]	3.1	12.2	11.3	68.9	4.5
Bronze ♂	7.6 ± 0.9	1.3	17.7	8.5	66.8	5.7
Wild-type ♀	8.1 ± 2.2	2.5	16.1	12.9	62.5	6.0
Bronze ♀	7.4 ± 1.7	2.7	15.0	9.0	68.4	4.9

[1] Mean ± Standard Deviation.

TABLE 3. Fatty acid composition of neutral lipids in wild-type *Aedes aegypti*

Lipid Fraction		Per Cent Composition of Fatty Acids								
		12	14	14:1	16	16:1	18	18:1	18:2	18:3
Triglyceride	♂	2.9	1.7	6.8	27.3	21.2	2.5	32.0	5.1	0.5
	♀	1.5	2.3	7.3	25.6	24.5	2.5	30.4	5.0	0.4
Mono- and diglyceride	♂	t[1]	3.0	3.7	29.6	15.9	5.3	28.3	9.9	4.1
	♀	t	4.5	3.8	34.2	17.0	6.8	24.0	7.4	2.1
Free fatty acid	♂	0.3	1.0	2.5	25.6	21.4	3.3	20.2	26.0	0.6
	♀	0.5	1.2	2.1	31.2	20.4	4.2	22.2	18.1	0.4
Sterol ester	♂	t	1.3	2.9	18.1	22.2	2.6	31.7	19.6	1.5
	♀	t	1.5	2.7	19.4	25.4	2.8	31.1	16.3	0.5

[1] t = trace, less than 0.1 per cent.

a greater number of components in this investigation and thus the per cent contribution of any single phosphatide is lessened. Khan and Brown (1966) and Fast and Brown (1961) both reported only cephalin (mostly phosphatidyl ethanolamine), lecithin (mostly phosphatidyl choline), and sphingolipid. The phospholipid composition as reported here is very similar to that of the housefly, *Musca domestica* (Crone and Bridges, 1963; Crone, 1964) and blowfly, *Phormia regina* (Bieber *et al.*, 1961).

The fatty acid composition of the various neutral lipid fractions in

TABLE 4. Fatty acid composition of neutral lipids in the mutant bronze of *Aedes aegypti*

Lipid Fraction		Per Cent Composition of Fatty Acids								
		12	14	14:1	16	16:1	18	18:1	18:2	18:3
Triglyceride	♂	0.5	1.9	10.5	27.6	18.4	2.7	28.5	9.4	0.2
	♀	0.7	2.0	7.1	28.9	20.1	3.2	29.4	8.4	0.3
Mono- and diglyceride	♂	t[1]	3.5	4.4	43.1	7.9	3.1	28.3	7.8	2.1
	♀	t	3.7	1.2	38.7	5.0	1.1	37.6	7.5	5.0
Free fatty acid	♂	0.3	0.7	0.6	72.6	2.7	15.4	4.7	3.6	t
	♀	0.2	2.7	1.7	68.4	1.0	21.4	3.3	1.5	t
Sterol ester	♂	t	0.8	2.5	20.7	20.1	1.8	25.8	26.6	1.7
	♀	t	0.8	1.9	22.5	19.2	3.3	20.9	29.7	1.6

[1] t = trace, less than 0.1 per cent.

wild-type and bronze mosquitoes is shown in Tables 3 and 4. The fatty acid composition of the wild-type is similar to the total lipid or neutral lipid fatty acid analyses of earlier workers (Barlow, 1964; Fast and Brown, 1962), but the bronze differs from the wild-type especially in the free fatty acid fraction. In this fraction the bronze contain considerably more of the saturated fatty acids C-16 (palmitic) and C-18 (stearic) than the wild-type strain. The higher amount of C-16 in the bronze is also apparent in mono- and diglycerides. There are also differences in the sterol ester fraction. The wild-type contains more C-18:1 (oleic) than C-18:2 (linoleic), while the reverse is true for the bronze.

The significance of the differences in fatty acid composition is not clear but the possible ramifications are interesting. Insect cuticular lipids contain free fatty acids (Bursell and Clements, 1967; Gilby and Cox, 1963) so perhaps the inferior water-proofing (Bhalla and Craig, 1967) of the bronze eggs may somehow be related to the free fatty acid differences in the bronze insect. This is especially significant since Bhalla and Craig emphasized that the egg shell is deposited by the maternal parent and hence represents the phenotype of the mother and not the offspring. Moreover, they have also shown through appropriate crosses that even the embryos with dark phenotypes within bronze shells die, thus indicating that the bronze females depositing the egg shells must have the metabolic deficiency. Bhalla and Craig hypothesized that a lack of melanin formation and failure of the egg shell to tan and harden might result in abnormal osmotic relations or in an imbalance of embryonic material and, subsequently, death. However, when they tanned the bronze eggs artificially with benzoquinone so the bronze eggs could not be differentiated from normal eggs, the embryos still died after a slight enhancement in development. This failure could have been due to a toxic effect of benzoquinone as suggested by the authors or possibly the fatty acid differences in the bronze mutant reported in this paper might be altering the water-proofing characteristics of the bronze egg. Another factor is the fatty acid complement provided to the embryo by the bronze female. Gilbert (1967) indicates that unsaturated fatty acids may be of great importance to the growth of insects. Jackson *et al.* (1968) suggest that a rather exact fatty acid composition is necessary for egg viability. Furthermore, they feel that the amount of fatty acid present as free fatty acid, methyl ester fatty acid, or sterol ester fatty acid may be equally important in determining egg viability. Thus, it is possible that the bronze female due to some metabolic alteration does not provide the embryo with the fatty acid composition necessary to complete embryonic development. This again could explain the failure of the artificial tanning to enhance egg hatching.

TABLE 5. Fatty acid composition of phospholipids in wild-type *Aedes aegypti*

Lipid Fraction		Per Cent Composition of Fatty Acids							
		14	16	16:1	18	18:1	18:2	18:3	>18:3
Lysophosphatidyl choline + sphingolipid	♂	7.6	31.8	14.7	9.5	22.1	14.3	t[1]	t
	♀	6.3	31.3	14.8	13.6	19.1	14.9	t	t
Phosphatidyl choline	♂	1.4	25.4	18.1	9.6	22.4	21.7	1.4	t
	♀	3.1	21.5	15.3	9.1	21.7	21.5	5.5	2.5
Phosphatidyl serine + phosphatidyl inositol + lysophosphatidyl ethanolamine	♂	t	17.0	22.4	8.7	19.7	32.1	t	t
	♀	t	12.9	25.0	5.5	14.7	31.8	6.4	3.4
Phosphatidyl ethanolamine	♂	1.9	23.4	8.9	6.8	26.9	32.1	t	t
	♀	2.6	25.4	12.2	6.3	26.8	24.4	2.8	t
Cardiolipin	♂	4.5	47.1	11.7	10.9	17.6	7.8	t	t
	♀	3.5	33.9	15.8	11.6	21.0	11.3	3.0	t

[1] t = trace, less than 1.0 per cent.

Fatty acid composition of the various phosphatides is shown in Tables 5 and 6. Here again the bronze mutant differs from the normal mosquito in that it contains considerably more of the saturated fatty acids (C-16 and

TABLE 6. Fatty acid composition of phospholipids in the mutant bronze of *Aedes aegypti*

Lipid Fraction		Per Cent Composition of Fatty Acids							
		14	16	16:1	18	18:1	18:2	18:3	>18:3
Lysophosphatidyl choline + sphingolipid	♂	6.6	32.5	13.9	13.1	19.5	14.3	t[1]	t
	♀	3.9	35.8	15.1	11.2	18.8	13.9	1.1	t
Phosphatidyl choline	♂	3.9	39.5	9.6	14.2	16.3	15.3	1.2	t
	♀	4.7	38.6	10.8	16.9	14.5	13.2	0.8	t
Phosphatidyl serine + phosphatidyl inositol + lysophosphatidyl ethanolamine	♂	8.8	40.0	13.1	13.6	17.2	4.1	3.4	t
	♀	1.8	36.1	16.1	11.3	18.6	11.4	4.5	t
Phosphatidyl ethanolamine	♂	7.3	47.5	10.2	14.6	13.1	4.5	2.7	t
	♀	1.6	36.0	11.3	9.1	23.3	17.3	1.3	t
Cardiolipin	♂	5.2	38.1	15.4	12.2	18.3	9.1	1.5	t
	♀	3.0	33.8	17.5	11.2	19.1	13.5	0.8	t

[1] t = trace, less than 1.0 per cent.

C-18) and less of the unsaturated. This is obvious in the phosphatidyl choline, phosphatidyl ethanolamine, and the phosphatidyl serine (plus other phosphatides) fractions. Phospholipids have been shown to play a variety of roles in a cell (Gilbert, 1967) including cell membrane structure, ion transport, active transport, mitochondrial activity, and nervous integration. Gilbert also feels that the low solubility of phospholipids in water makes them ideal substances for use in membranes that are vital for both the partitioning of separate cells and the micro-compartmentalization of the cytoplasm. Thus, it is possible that the differences in fatty acid composition in bronze phospholipids may be responsible for the improper osmotic relations suggested by Bhalla and Craig (1967).

In summary, the bronze mosquito differs from wild-type *Aedes aegypti* in that it possesses more saturated fatty acids and less unsaturated fatty acids in a number of the neutral lipid and phospholipid fractions. These findings do not explain the failure of the bronze eggs to complete embryonic development, but an alteration in fatty acid metabolism in the bronze female could be at least partially responsible since previous work indicates that fatty acids (1) are components of cuticular lipids, (2) are thought to be necessary in specific amounts for egg viability, and (3) are integral parts of phospholipid molecules. Further work, including lipid analyses of eggs and fatty acid tracer experiments using a number of different strains of this mosquito, is necessary to explain sterility in the mutant bronze.

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